

WHAT IS CLAIMED AS NEW AND INTENDED TO BE COVERED BY
LETTERS PATENT OF THE UNITED STATES IS:

1. A method of detecting in a sample an analyte
(A) having a molecularly recognizable portion thereon,
which comprises:

providing a molecular bridging entity (B) having
thereon:

- (i) a portion capable of recognizing
said molecularly recognizable
portion on said analyte; and
- (ii) a portion comprising a
polynucleotide sequence; and

(C) a signalling entity having thereon:

- (i) a polynucleotide portion capable
of annealing to said
polynucleotide portion of said
bridging entity, thereby to form a
stable polynucleotide hybrid, and
- (ii) a signal generating portion;

forming a complex comprising:

- (1) said analyte (A) complexed through
said molecularly recognizable
portion to
- (2) said recognizing portion of said
entity (B); said entity (B) being

complexed through said polynucleotide portion thereof to
(3) said polynucleotide portion of
said signalling entity (C); and

detecting a signal by means of said signal
generating portion present in said complex.

2. The method of Claim 1 wherein said analyte is
present in a biological or non-biological sample.

3. The method of Claim 1 wherein said molecularly
recognizable portion on said analyte is proteinaceous.

4. The method of Claim 1 wherein the molecularly
recognizable portion on said analyte comprises nucleic
acid.

5. The method of Claim 1 wherein the molecularly
recognizable portion on said analyte comprises a
saccharide.

6. The method of any of Claims 3, 4 or 5 wherein
said analyte is selected from the group consisting of
an antigen, an antibody, a receptor, a virus, a viral
component, a bacterium, a bacterial component, a cell,
a cellular component, or any pathogenic or non-
pathogenic component of a sample.

7. The method of Claim 1 wherein said recognizing
portion on said bridging entity comprises a
polynucleotide sequence.

8. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises an antigen.

9. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises an antibody.

10. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a saccharide.

11. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a lectin.

12. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a hormone.

13. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a receptor.

14. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises an enzyme inhibitor or enzyme cofactor.

15. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises an enzyme active site, a cofactor binding site, or a receptor protein.

16. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof.

17. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity does not code for a gene sequence or fragment thereof.

18. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity comprises a poly deoxy G, poly deoxy C, poly deoxy T or poly deoxy A sequence, or any poly-ribo or -deoxyribo purine, pyrimidine or analog.

19. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity comprises a sequence portion which is rich in guanosine residues.

20. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to another polynucleotide sequence.

21. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to an antibody.

22. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to an antigen.

23. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to a saccharide.

24. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to a lectin.

25. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to a hormone.

26. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to a receptor.

27. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to an enzyme inhibitor or enzyme cofactor.

28. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to an enzyme.

29. The method of Claim 7 wherein said bridging entity is a circular DNA polymer.

30. The method of Claim 29 wherein said DNA is single-stranded.

31. The method of Claim 29 wherein said circular DNA polymer is derived from a filamentous phage.

32. The method of Claim 31 wherein said filamentous phage is M13 or a variant thereof.

33. The method of Claim 32 wherein said M13 phage carries a sequence portion which is rich in guanosine residues, or cytosine residues.

34. The method of Claim 1 wherein said polynucleotide portion on said signalling entity codes for a gene product or fragment thereof.

35. The method of Claim 1 wherein said polynucleotide portion on said signalling entity does not code for a gene product or fragment thereof.

36. The method of Claim 1 wherein said polynucleotide portion on said signalling entity comprises a poly deoxy C, poly deoxy G, poly deoxy A, poly deoxy T sequence, or a repeating sequence of low complexity.

37. The method of Claim 1 wherein said polynucleotide portion on said signalling entity comprises a sequence portion which is rich in cytosine residues, or guanosine residues.

38. The method of Claim 1 wherein said signalling entity is a polynucleotide polymer.

39. The method of Claim 38 wherein said polynucleotide polymer is a naturally occurring modified DNA.

40. The method Claim 39 wherein said polynucleotide polymer is derived from a T (even) phage.

41. The method of Claim 40 wherein said T (even) phage is T₄.

42. The method of Claim 39 wherein said modified DNA carries a cloned insert.

43. The method of Claim 38 wherein said polymer is single-stranded.

44. The method of Claim 43, wherein said polymer is derived from a filamentous phage.

45. The method of Claim 44 wherein said phage is M13 or a variant thereof.

46. The method of Claim 1 wherein said signal generating portion of said signalling entity is radiolabeled.

47. The method of Claim 1 wherein said signal generating portion of said signalling entity is not radiolabeled.

48. The method of Claim 47 wherein said signal generating portion comprises an enzyme.

49. The method of Claim 47 wherein said signal generating portion comprises a biotin moiety.

50. The method of Claim 47 wherein said signal generating portion comprises a fluorogenic compound.

51. The method of Claim 47 wherein said signal generating portion comprises an electron dense compound.

52. The method of Claim 47 wherein said signal generating portion comprises or binds to an insoluble phase.

53. The method of Claim 52 wherein said insoluble phase comprises a latex particle, a resin, or a bacterium.

54. The method of Claim 47 wherein said signal generation portion comprises an antibody or antigen.

55. The method of Claim 47 wherein said signal generating portion comprises a saccharide or lectin.

56. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises a radioactivity measurement.

57. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises an enzymatic reaction.

58. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises a fluorescence measurement, or electron microscopic measurement.

59. The method of Claim 47 wherein said signal generating portion is a polynucleotide sequence capable of recognizing a signal containing moiety.

60. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises an antibody/antigen complexation reaction.

61. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises a complexation reaction between biotin and a biotin binding moiety.

62. The method of Claim 61 wherein said moiety is avidin, streptavidin or an anti-biotin antibody.

63. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises detection of an electron dense compound.

64. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises a complexation reaction between a saccharide and a lectin.

65. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises a binding step on an insoluble phase.

66. The method of Claim 1 wherein said step of detecting a signal by means of said signal generating portion comprises complexation between a signalling entity comprising a cloned insert on a naturally occurring modified DNA, and the bridging moiety, followed by binding a modified lectin to said signalling entity.

67. The method of Claim 66 wherein said modified DNA is derived from a T₄ phage.

68. The method of Claim 65 wherein said insoluble phase is a latex particle.

69. The method of Claim 1 wherein said recognizable portion on said analyte is a

polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating portion on said signalling entity is based on non-radioactive detection.

70. The method of Claim 69 wherein said bridging entity is derived from a filamentous phage.

71. The method of Claim 69 wherein said signalling entity is derived from a filamentous phage.

72. A polynucleotide sequence covalently attached to an antibody.

73. The sequence of Claim 72 wherein said antibody is monoclonal.

74. A polynucleotide sequence covalently attached to a lectin.

75. A polynucleotide sequence covalently attached to a saccharide having up to 20 saccharide units.

76. A polynucleotide sequence covalently attached to receptor.

77. A polynucleotide sequence covalently attached to a hormone.

78. A DNA molecule carrying a polynucleotide portion which comprises a sequence selected from the group consisting of poly dGT, poly dAC, poly dCT, poly

dAT, poly dGC, poly dGA, poly dG, poly dC, poly dT, poly dA, and a repeating low-complexity polynucleotide.

79. The DNA molecule of Claim 78 which is a filamentous phage.

80. The phage of Claim 79 which is M13 or a variant thereof.

81. The DNA molecule of any of Claims 78 or 79 wherein said sequence is at least an oligonucleotide.

82. The DNA molecule of any of Claims 78 or 79 which also carries a polynucleotide sequence complementary to part of whole of a gene sequence of a nucleic acid-containing organism.

83. The DNA molecule of Claim 82 wherein said organism is a virus, a prokaryotic or a eukaryotic cell.

84. The DNA molecule of Claim 83 wherein said prokaryotic cell is a bacterium.

85. The DNA molecule of Claim 83 wherein said eukaryotic cell is a mammalian cell.

86. The DNA molecule of Claim 82 which is, a filamentous phage.

87. The DNA molecule of Claim 82 which is M13 or a variant thereof.

88. A circular DNA molecule covalently attached to a non radiolabelled signal generating moiety.

89. The DNA molecule of Claim 88 which is a filamentous phage.

90. The DNA molecule of any of Claims 88 or 89 which carries a polynucleotide portion which comprises a sequence selected from the group consisting of poly dGT, poly dAC, poly dCT, poly dAT, poly dGC, poly dGA, poly dG, poly dC, poly dT, poly dA and a repeating low-complexity polynucleotide.

91. The DNA molecule of any of Claims 88 or 89 which carries a polynucleotide portion which is rich in cytosine residues.

92. The DNA molecule of Claim 90 wherein said sequence is an oligonucleotide.

93. The DNA molecule of any of Claims 88 or 89 which carries a polynucleotide portion which comprises a sequence coding for part or whole of a gene.

94. The DNA molecule of any of Claims 88 or 89 wherein said signal generating moiety comprises a radiolabel.

95. The DNA molecule of any of Claims 88 or 89 wherein said signal generating moiety is non-radiolabeled.

96. The DNA molecule of Claim 93 wherein said signal generating moiety comprises an enzyme.

97. The DNA molecule of Claim 93 wherein said signal generating moiety comprises a biotin moiety.

98. The DNA molecule of Claim 93 wherein said signal generating moiety comprises an antibody.

99. The DNA molecule of Claim 93 wherein said signal generating moiety comprises a fluorogenic compound.

100. A kit useful for the detection of an analyte (A) having a molecularly recognizable portion thereon, comprising:

- I) a carrier being compartmentalized to receive in close confinement therein one or more container means;
- II) a first container means containing a molecular bridging entity (B) having thereon:
 - (i) a portion capable of recognizing said molecularly recognizable portion on said analyte (A); and
 - (ii) a portion comprising a polynucleotide sequence; and
- (III) a second container means containing a signalling entity (C) having thereon:
 - (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity (B) thereby to

form a stable polynucleotide
hybrid; and

(ii) a signal generating portion.

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101. The kit of Claim 100 which also comprises

IV) a third container means containing
components needed to detect a signal from
said signal generating means.

102. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises a
polynucleotide sequence.

103. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises
an antigen.

104. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises
an antibody.

105. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises a
saccharide.

106. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises a
lectin.

107. The kit of Claim 100 wherein said
recognizing portion on said bridging entity comprises a
hormone.

108. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises a receptor.

109. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises an anzyme inhibitor or enzyme cofactor.

110. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises an enzyme active site or cofactor binding site.

111. The kit of Claim 100 wherein wherein said polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof.

112. The kit of Claim 100 wherein said polynucleotide sequence on said bridging entity does not code for a gene product or fragment thereof.

113. The kit of Claim 100 wherein said polynucleotide sequence on said bridging entity comprises a poly dG, poly dC, poly dT, poly dA sequence, or a low complexity (repeating) polynucleotide.

114. The kit of Claim 100 wherein said polynucleotide sequence on said bridging entity comprises a sequence portion which is rich in guanosine residues.

115. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to another polynucleotide sequence.

116. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to an antibody.

117. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to an antigen.

118. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to a saccharide.

119. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to a lectin.

120. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to a hormone.

121. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to a receptor.

122. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to an enzyme inhibitor or enzyme cofactor.

123. The kit of Claim 100 wherein said polynucleotide sequence in said bridging entity is covalently attached to an enzyme.

124. The kit of Claim 100 wherein said bridging entity is a circular DNA polymer.

125. The kit of Claim 124 wherein said circular DNA is single-stranded.

126. The kit of Claim 125 wherein said circular DNA polymer is derived from a filamentous phage.

127. The kit of Claim 124 wherein said filamentous phage is M13 or a variant thereof.

128. The kit of Claim 125 wherein said M13 phage carries a sequence portion which is rich in guanosine or cytosine residues.

129. The kit of Claim 100 wherein said polynucleotide portion on said signalling entity codes for a gene product or fragment thereof.

130. The kit of Claim 100 wherein said polynucleotide portion on said signalling entity does not code for a gene product or fragment thereof.

131. The kit of Claim 100 wherein said polynucleotide portion on said signalling entity comprises a poly dC, poly dG, poly dA, poly dT sequence, or a low-complexity, repeating polynucleotide.

132. The kit of Claim 100 wherein said polynucleotide portion on said signalling entity comprises a sequence portion which is rich in cytosine or guanosine residues.

133. The kit of Claim 100 wherein said signalling entity is a circular DNA polymer.

134. The kit of Claim 133 wherein said DNA is single-stranded.

135. The kit of Claim 134 wherein said DNA is derived from a filamentous phage.

136. The kit of Claim 135 wherein said phage is M13 or a variant thereof.

137. The kit of Claim 100 wherein said signal generating portion on said signalling entity is radiolabeled.

138. The kit of Claim 100 wherein said signal generating portion of said signalling entity is not radiolabeled.

139. The kit of Claim 138 wherein said signal generating portion comprises an enzyme.

140. The kit of Claim 138 wherein said signal generating portion comprises a biotin moiety.

141. The kit of Claim 138 wherein said signal generating portion comprises a fluorogen.

142. The kit of Claim 138 wherein said signal generating portion comprises an electron dense compound.

143. The kit of Claim 138 wherein said signal generating portion comprises or binds to an insoluble phase.

144. The kit of Claim 138 wherein said insoluble phase comprises a latex particle, a resin, or a bacterium.

145. The kit of Claim 138 wherein said signal generating portion comprises an antibody.

146. The kit of Claim 138 wherein said signal generating portion comprises a saccharide.

147. The kit of Claim 100 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said signal generating portion on said signalling entity is based on non-radioactive detection.

148. The kit of Claim 147 wherein said bridging entity is derived from a filamentous phage.

149. The kit of Claim 147 wherein said signalling entity is derived from a filamentous phage.

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Add C1

Add D1

Add E15

Add F4

Add G4